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DToL Tissue and Blood Sampling Standard Operating Procedure: Chordata: Vertebrata: Aves V.1

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Inez Januszczak[1], Nancy Holroyd[2]

[1]Natural History Museum; [2]Wellcome Sanger Institute

Darwin Tree of Life

Inez Januszczak
Natural History Museum, London

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This is a working protocol and may be subject to change.

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Abstract

This Standard Operating Procedure (SOP) contains guidance on how to sample tissue and blood from bird specimens submitted to the Darwin Tree of Life (DToL) project.

Please note this SOP covers sampling tissue and blood either during an appropriate veterinary procedure or **post-mortem,** with assumed veterinary knowledge.

It is applicable if the submitter/centre in question has opportunities to sample tissue from birds that are being either being euthanized for clinical purposes, undergoing veterinary procedures. or eligible biobanked carcasses (storage dependent).

This SOP does not cover procedures regarding bio banked, pre - dissected tissue or blood.

This SOP is covers birds specifically - please refer to the **DToL Chordata: Vertebrata** SOP for a general overview.

For vouchering and sampling fish specimens, with the idea of donating tissue and carcasses specifically to the Natural History Museum in London, please refer to the **DToL Chordata: Vertebrata: Fish Vouchering SOP**.

This is version one of this SOP, and may be subject to change in the future.

Attachments

XLSX

DToL Specimen submis...

18KB

Guidelines

Tissue/blood sampling post mortem

If the centre in question has opportunities to sample tissue and blood from birds that are being either being euthanized for clinical purposes, undergoing veterinary procedures or biobanked carcasses, such samples would be very useful for the Darwin Tree of Life (DToL) project.

Regulatory compliance considerations

Animal Welfare - the samples will only come from birds undergoing veterinary care, euthanized for health reasons or biobanked carcasses that are eligible for dissection/ tissue preparation.

Permissions may be requested depending on the species in question.

Note

Regarding frozen, whole carcasses

Please note the ideal storage conditions for a biobanked carcasses will be at -20 degrees or lower. Carcasses that have gone through multiple types of preservation should be handled on a case by case basis - please contact the Natural History Museum (NHM) and Sanger Sampling teams, listed at the end of the SOP.

Carcasses stored in ethanol (at room temperature or lower) will only be considered in exceptional circumstances, for example if it is a rare species.

Please note: If the frozen carcass is in exceptional condition, or of a rare or unusual species, the NHM will consider obtaining the carcass and performing the dissection. This is to comply with the voucher/ skin requirements of the NHM Birds curatorial staff.

Similarly if the submitter in question does not have dissecting facilities but does have a frozen carcass of a specimen of interest, the NHM will consider doing the dissection separately and liaise with the Sanger Institute on the behalf of the submitter.

This will require the submitted carcass to NOT be dissected and instead sent to the museum or to a relevant partner whole, and still frozen. The NHM could provide postage and shipping facilities to do this. Do enquire with the NHM sampling team and Sanger sampling team as appropriate (email addresses at the bottom of the SOP).

Logistical Considerations

A -80 freezer is required for tissue sampling (suggested brand Fryka B35-85 under bench, ETA 2-3 weeks 80kg. 58×76×54cm). Preserving the tissue directly on dry ice is also an option.

Prior to submission

1. It is important to check whether DToL would require the sample.

The submitter/centre in question would be shared a species target list. As this is opportunistic sampling, the list should be regularly checked to see what species are needed.

Note

Please ensure each submitted specimen has available all its relevant metadata (Date of Collection, Collector, Identifier, Collection location, Species, Specimen ID).

The Darwin Tree of Life project will **not** accept specimens which don't have this information.

2. Sampling

Aside from the specimen itself, labelled cryotubes that are able to withstand -80 degrees cold storage will be required (for the tissue) as well as ziplock/ plastic bags to store the tubes and carcasses.

Each specimen requires two identical ID numbers; one will be used to label the ziplock bag of tubes containing the samples, the other to label the remaining carcass (if applicable).

The NHM is able to provide unique ID numbers, otherwise any matching ID will be appropriate, preferably a series of numbers.

The NHM is able to provide cryotubes with unique IDs if this aids the archiving.

Note

Frozen carcasses will need to be partially defrosted prior to tissue preparation. The most frozen part of the tissue should thus be removed and preserved.

If the specimen (in any of the listed scenarios) is suitable:

a. Take a photo of the specimen – a dorsal and ventral photograph, with the specimen ID clearly visible in the frame of the photo. This photo will act as a photographic voucher if it is not possible to retain to the rest of the carcass post dissection.

b. Dissect out multiple (ideally minimum of 3, up to 8) samples per tissue below, maximum 0.5g per tube (around pea-sized) as is practical from the size and condition of specimen:

Inject out blood IF POSSIBLE

Muscle
Heart
Liver
Brain
Gonads

Please note listed tissues are ranked in order of priority.

Frozen carcasses will need to be partially defrosted prior to tissue preparation. The most frozen part of the tissue should thus be removed and preserved.

Note

Regarding blood – 200ul is the ideal amount, as it provides enough material for HiC, PacBio and RNA.
25ul is the minimum required.

After the blood is removed, it must be flash frozen at -80 degrees with molecular grade ethanol (100%).

If this is not possible, flash frozen without ethanol is preferred.

If flash freezing the blood is not an option, it can be stored in ethanol only (molecular grade).

c. Put the tissue immediately into clearly labelled tube (with a unique tube ID) and place immediately into the -80 freezer/dry ice. Place all tubes into a ziplock bag along with tissue information- keeping tissues separate can also help with the labeling. Ensure each ziplock bag of samples has its own specimen ID label.

The NHM is able to provide tubes with unique IDs if this helps with the archiving.

d. Put the rest of the carcass into a ziplock bag, double bag the carcass, then label with the second identical specimen ID.

e. Add the appropriate data to an Excel spreadsheet, clearly linking the specimen ID, tube barcode and tissue type. A draft spreadsheet is attached to this protocol.

f. Email the DToL NHM sampling coordinator regarding the samples obtained.

The NHM is interested in obtaining the rest of the carcass as a specimen voucher, regardless of its condition after post-mortem. If it is possible to store the rest of the carcass in minimum –20 conditions, dry frozen, then it should be stored. The carcass must thus also remain frozen throughout its transportation to the museum.

3. Submission

Once the submitter/center has collected at least 5 sets of samples from 5 specimens, and thus potentially 5 carcasses, they should contact NHM and Sanger DToL Sampling teams who will arrange for their collection.

Note

Please note - an Excel spreadsheet with Specimen ID, Species, tube ID and tissue type of all submitting samples must also be kept (including whether a carcass was kept or not).

Each tube ID must match the tissue sampled.

Ensure that samples from separate animals are kept separate.

This spreadsheet must also be emailed to the NHM and Sanger DToL Sampling team. Any photographs of the specimens must also be saved and emailed to the DToL NHM Sampling coordinator.

When submitting/ considering a submission, please contact:

Inez Januszczak, DToL NHM Sampling Coordinator: **Inez.januszczak@nhm.ac.uk**
The DToL NHM Sampling team: **darwintreeoflife@nhm.ac.uk**
Molly Carter, DToL Sanger/Tree of Life Coordinator: **mc39@sanger.ac.uk**

Troubleshooting

Safety warnings

Please note - it is assumed all appropriate zoonotic disease checks have been carried out prior to any post mortem or veterinary procedure.

Ethics statement

Regulatory Compliance Considerations

Animal Welfare - samples will only be accepted if they come from animals undergoing veterinary care. euthanized for health reasons or from a valid biobank collection.